Cell Metabolism
Short Article

PPARγ in Vagal Neurons Regulates High-Fat Diet Induced Thermogenesis

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SUMMARY

The vagus nerve innervates visceral organs providing a link between key metabolic cues and the CNS. However, it is not clear whether vagal neurons can directly respond to changing lipid levels and whether altered "lipid sensing" by the vagus nerve regulates energy balance. In this study, we systematically profiled the expression of all known nuclear receptors in laser-captured nodose ganglion (NG) neurons. In particular, we found PPARγ expression was reduced by high-fat-diet feeding. Deletion of PPARγ in Phox2b neurons promoted HFD-induced thermogenesis that involved the reprograming of white adipocyte into a brown-like adipocyte cell fate. Finally, we showed that PPARγ in NG neurons regulates genes necessary for lipid metabolism and those that are important for synaptic transmission. Collectively, our findings provide insights into how vagal afferents survey peripheral metabolic cues and suggest that the reduction of PPARγ in NG neurons may serve as a protective mechanism against diet-induced weight gain.

INTRODUCTION

The obesity epidemic is spreading at an accelerated pace, currently affecting more than 500 million people around the world. While it is well appreciated that excessive nutrients from a fat- and carbohydrate-rich diet are responsible for obesity and its comorbid conditions (Unger and Scherer, 2010), much of how nutritional status is regulated at the organismal level remains to be understood. The metabolic status of visceral organs is conveyed in part by circulating hormones such as leptin and insulin that can act directly upon discrete neural pathways in the central nervous system (CNS) to regulate energy balance (Myers and Olson, 2012). Notably, a second and less-appreciated pathway exists involving direct neuronal innervation by sensory afferents of the spinal and vagus nerves. In particular, vagal sensory neurons in the nodose ganglion (NG) densely innervate key visceral organs such as the liver, portal vein, and the gastrointestinal (GI) tract. Moreover, these neurons send ascending projections to the nucleus of solitary tract (NTS) in the brain where incoming visceral information is further processed and integrated (Berthoud, 2008). Therefore, it has long been hypothesized that vagal sensory neurons are anatomically positioned to serve as a link between changing levels of peripheral metabolic cues and the CNS pathways that control energy homeostasis.

Accumulating evidence arising from physiological (Chi and Powley, 2007; Tellez et al., 2013; Wang et al., 2008), electrophysiological (Lal et al., 2001; Randich et al., 2000), and biochemical (de Lartigue et al., 2011; Duca et al., 2013; Paulino et al., 2009) studies have established that vagal sensory neurons respond to both acute and long-term changes in nutritional status including that of dietary-derived lipids. The acute response of vagal afferents to intestinal lipids are thought to be regulated by gut peptides and neurotransmitters that are transiently released from the neighboring enteroendocrine cells (Glatzle et al., 2003; Raybould et al., 1998; Moran et al., 1997). However, it remains unclear whether vagal sensory neurons themselves have the capacity to directly sense peripheral lipid status and whether altered lipid sensing by the vagus nerve can impair long-term energy and glucose homeostasis.

To identify candidate genes and pathways that may play a role in the vagal control of lipid sensing, we purified RNA from vagal sensory neurons in the NG and subjected them to genome-wide gene expression analysis. Our profiling studies of NG neurons revealed an unexpected enrichment of nuclear receptors (NRs) in particular, peroxisome proliferator-activated receptor γ (PPARγ), that are ligand-activated transcription factors and receptors for many dietary-derived lipids (Chawla et al., 2001). The enrichment of several "lipid sensors" such as PPARγ in NG neurons therefore raised the possibility that NRs in vagal sensory neurons modulate energy balance by directly sensing changing levels of peripheral metabolic cues. Despite its well-documented role in lipid metabolism in peripheral organs, PPARγ function in vagal sensory neurons remains to be established. In the current study, we investigated the physiological role of PPARγ by genetically ablating its expression in vagal neurons. Our findings revealed a role for PPARγ in vagal sensory neurons in sensing and relaying peripheral lipid status toward the CNS and highlight the role of the vagus nerve as a critical site where PPARγ modulates energy homeostasis.

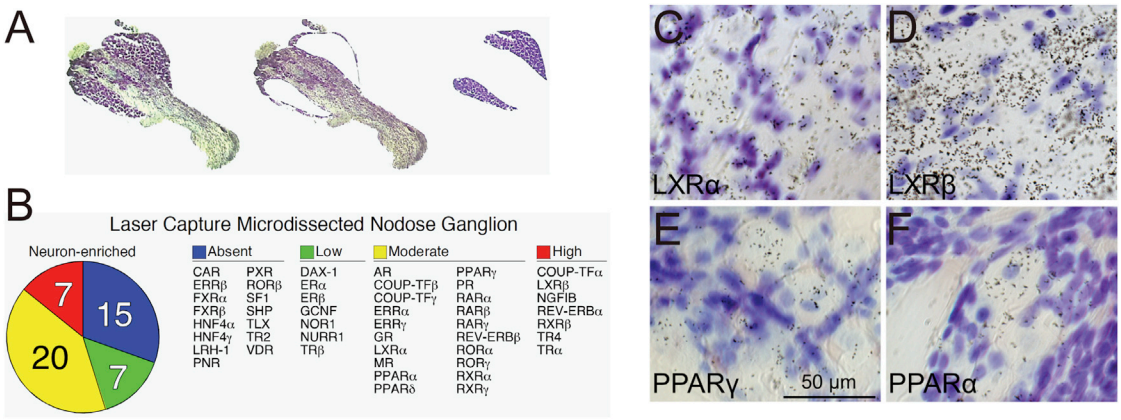

Figure 1. NR Expression in the NG

(A) Laser-guided microdissection of neuron-enriched samples from thionin-counterstained nodose ganglia. Neuron-enriched areas appear darkly counterstained, as opposed to unstained regions, which contain mostly fibers and glial cells.

(B) Relative levels of NR expression are indicated by the pie chart and shown in the tables to the right.

(C–F) In situ hybridization of select lipid-sensing NRs in the NG. Clusters of silver grains (^{35}S) indicate mRNA expression of LXRα (C), LXRβ (D), PPARγ (E), and PPARα (F).

RESULTS

Profiling Nuclear Receptor Expression in the NG

We have previously established a high-throughput quantitative real-time PCR (qPCR) assay to examine NR expression in peripheral tissues and in the CNS (Bookout et al., 2006). Using this approach, we investigated the expression patterns of all known murine NRs in the NG of male C57BL/6 mice. RNA was extracted from the left NG, in which the residing vagal sensory neurons predominantly innervate metabolically important abdominal viscera (e.g., pancreas, GI tract, and liver). In order to minimize RNA contamination from the epineurium of peripheral nerves, cell bodies of NG neurons were isolated by laser capture microdissection (Figure 1A). Our analysis identified transcripts of 34 NRs (out of the 49 known NRs) in the NG (Figure 1B). Notably, we found several NRs that are important for lipid metabolism and energy balance were expressed at moderate to high levels in NG neurons (Figure 1B). These include glucocorticoid receptor, liver X receptor-α, and liver X receptor-β (LXRα and LXRβ), which act in a variety of cell types, including CNS neurons to control distinct aspects of nutrient metabolism (Chawla et al., 2001). Moreover, PPARα and PPARγ, known as dietary lipid sensors in the liver and adipose tissue, respectively, were expressed in the NG (Figure 1B). Furthermore, members of the retinoid X receptor family, which act as heterodimer partners to PPARs (Chawla et al., 2001), were also expressed in the NG (Figure 1B). Expression of selected NRs in the NG was further verified by in situ hybridization with ^{35}S-labeled riboprobes (Figures 1C–1F).

High-Fat-Diet Feeding Reduces PPARγ Expression in the NG

NRs directly bind dietary-derived lipids to alter target gene expression in response to internal and external cues (Chawla et al., 2001). We investigated whether PPARγ expression in the NG was nutritionally regulated by challenging the mice with HFD (42% kcal from fat). Eight-week-old C57BL/6 mice were fed ad libitum on either regular chow (4% kcal from fat) or HFD for a total of 14 weeks before being sacrificed for gene expression analysis. We found that the expression of PPARγ mRNA was significantly reduced in NG neurons after chronic exposure to HFD (Figure 2A), whereas levels of PPARδ as well as the two LXR receptors (LXRα and LXRβ) remained unchanged (Figures 2B–2D). We further examined whether a short-term exposure to HFD would result in a similar reduction of these receptors in NG neurons. Remarkably, levels of PPARγ decreased after only 5 days of HFD feeding (Figure 2E). In comparison, its levels in white adipose tissue (WAT) and brown adipose tissue (BAT) remained constant during this period (Figure S1 available online). Notably, several canonical transcriptional targets of PPARγ that are important for lipid metabolism were also present in the NG. Their levels were significantly reduced after 5 days of HFD feeding (Figure 2F) and remained suppressed after chronic HFD exposure (data not shown). Interestingly, we found that HFD reliably alters the expression PPARγ and its transcriptional targets in NG neurons. However, treatment with the PPARγ selective agonist, rosiglitazone, did not (Figures S1D–S1G). This finding suggests that HFD and rosiglitazone may activate PPARγ through distinct mechanisms to modulate different cellular functions in NG neurons (Choi et al., 2010).

Ablation of PPARγ in *Phox2b* Neurons

The downregulation of PPARγ in the NG neurons after HFD feeding raised the intriguing possibility that it played a role in mediating HFD-induced metabolic alterations. One critical way to test this hypothesis is to selectively ablate PPARγ function in vagal afferents and investigate whether this contributes to HFD-induced thermogenesis and/or metabolic syndrome. While PPARγ function has been characterized in several peripheral organs, its expression pattern and function in the nervous system has not been well studied. To this end, we first assessed PPARγ expression in laser-captured neurons collected from distinct neuronal populations in the peripheral and central

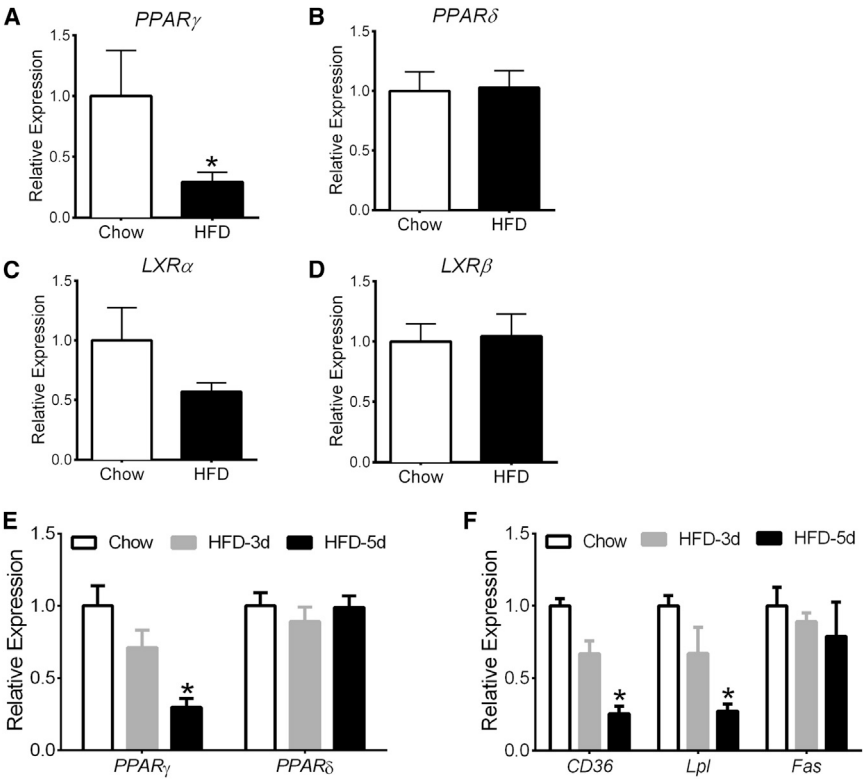

Figure 2. NR Expression in Nodose Neurons after Long- and Short-Term Feeding with HFD

(A–D) Relative mRNA levels of PPARγ (A), PPARδ(B), LXRα(C), and LXRβ(D) in the NG after 14 weeks of feeding with either chow (n = 6) or HFD (n = 8). Error bars show SEM. The symbol * indicates $p < 0.05$.

(E and F) Relative gene expression in the NG after 3-day and 5-day feeding with either chow (n = 10) or HFD (n = 10). Error bars show SEM. * indicates $p < 0.05$.

nervous systems. Among the sites examined, PPARγ was most abundantly expressed in the NG, whereas its expression was markedly lower in the spinal dorsal root ganglion (Figure S2A). In the brain, our analysis detected strong PPARγ expression in the cerebral cortex and the organum vasculosum of the lamina terminalis (Figure S2B), a pattern that is consistent with previous report (Gofflot et al., 2007) and with our own observations by in situ hybridization (data not shown). Notably, PPARγ was present at low levels in the NTS, a target of incoming vagal sensory information, whereas it was not detectable in the dorsal motor nucleus of the vagus, the parasympathetic motor output of the vagus nerves (Figure S2B).

To ablate PPARγ function in vagal sensory neurons, we bred floxed PPARγ mice (He et al., 2003) with mice that express bacteriophage-derived Cre recombinase driven by the regulatory sequences of the *Phox2b* gene (Scott et al., 2011). *Phox2b* is a homeodomain transcription factor that plays an essential role in the differentiation of the visceral nervous system (Pattyn et al., 1999). We have previously generated and characterized a transgenic *Phox2b*::*Cre* line that showed restricted Cre expression in the peripheral nervous system (Rossi et al., 2011). Cre activity was detected in all neurons in the NG but was absent from other sympathetic or parasympathetic ganglia (Gautron et al., 2013; Scott et al., 2011). Notably, *Phox2b*::Cre was present in the second-order visceral sensory neurons in the NTS. Therefore, using the *Phox2b*::Cre allows for the ablation of PPARγ function in both central and peripheral branches of the vagal afferents.

Removal of the floxed exons of PPARγ gene generates truncated and nonfunctional PPARγ transcripts that can be detected by PCR. We therefore assessed the expression of the full-length as well as the truncated PPARγ transcripts in peripheral tissues and in multiple brain sites of Phox2b::Cre; $PPAR\gamma^{fl/fl}$ mice. Cre-mediated ablation could only be detected in the NG and in the brainstem. Recombination was not found in any forebrain structures nor in other peripheral organs that we examined (Figure S2D). We further assessed the levels of full-length PPARγ transcripts in the NG using a Taqman probe that didn't recognize the truncated isoforms. Our analysis revealed that PPARγ mRNA expression was comparable between $PPAR\gamma^{fl/fl}$ and Phox2b::Cre; $PPAR\gamma^{fl/+}$ mice, whereas their levels were significantly reduced in Phox2b::Cre; $PPAR\gamma^{fl/fl}$ mice (Figure S2C).

PPARγ in *Phox2b* Neurons Regulates Body Weight and Composition

To determine whether PPARγ function in the vagal afferents is required for normal energy homeostasis, we conducted metabolic analysis using Phox2b::Cre; $PPAR\gamma^{fl/fl}$ (designated hereafter as $PPAR\gamma^{Phox2b\ KO}$) mice and their littermate controls (Phox2b::Cre; $PPAR\gamma^{fl/+}$). We found no significant differences in glycemia or lipidemia between $PPAR\gamma^{Phox2b\ KO}$ mice and their littermate controls (Figures S3A–S3J). Moreover, chow-fed $PPAR\gamma^{Phox2b\ KO}$ mice initially had comparable body weights to their littermate controls until 13 weeks of age. However, body weights began to diverge at 14 weeks of age as $PPAR\gamma^{Phox2b\ KO}$ mice gained less weight and became significantly leaner than their littermates at 17 weeks of age (Figure S4A). This lean phenotype was more prominent when $PPAR\gamma^{Phox2b\ KO}$ mice were fed on HFD, as their body weights diverged much earlier and faster (Figures S4B and S4D). Under both dietary conditions, nuclear magnetic resonance analysis revealed that the decreased body weight in $PPAR\gamma^{Phox2b\ KO}$ mice was due to a reduction in the fat mass, but not in the lean mass (Figure S4C).

We probed whether the decreased body weight in $PPAR\gamma^{Phox2b\ KO}$ mice was the result of a reduction in food intake. Surprisingly, in weight-matched mice, we found no differences in the cumulative food intake over a 5 day period (Figure S4G). Moreover, daily intake during the light and dark cycles was comparable between $PPAR\gamma^{Phox2b\ KO}$ mice and their

littermate controls (Figure S4H). We further subjected mice to a "refeeding" paradigm in which food intake was measured after an overnight fast and found no differences between the genotypes (Figure S4I).

PPARγ in *Phox2b* Neurons Regulates Energy Expenditure

To further investigate the lean phenotype, a new cohort of PPARγ$^{Phox2b\ KO}$ mice and their littermate controls were assessed in the metabolic chambers. All experiments were conducted in weight-matched male mice before differences in body weight became evident (Figure 3A). Despite the divergence of body weight at a later stage, indirect calorimetry analyses failed to detect significant differences in energy expenditure (EE) between chow-fed PPARγ$^{Phox2b\ KO}$ and their littermate controls when these mice were tested at 11 weeks of age (Figures 3B–3D). However, during a food-restriction challenge, PPARγ$^{Phox2b\ KO}$ lost more weight than their littermate controls (Figures S4E and S4F), suggesting that these mice had higher EE during the period of negative energy balance.

Increased EE was more evident when PPARγ$^{Phox2b\ KO}$ mice were fed HFD (Figures 3A–3D). These mice demonstrated significantly elevated levels of oxygen consumption (Figure 3B), carbon dioxide, and heat production (Figures 3C and 3D) when housed in the metabolic chambers. An increase in physical activity was also observed in PPARγ$^{Phox2b\ KO}$ mice (Figure 3F), whereas the respiratory exchange ratio remained unchanged (Figure 3E). The HFD-exacerbated increase in EE was best demonstrated when weight-matched PPARγ$^{Phox2b\ KO}$ mice and control littermates were acutely exposed to HFD. A counter-regulatory increase in EE normally follows the switch from regular chow diets to HFD, and this effect was exacerbated in the PPARγ$^{Phox2b\ KO}$ mice. Before the switch, the mice were maintained on regular chow, and EE was comparable between PPARγ$^{Phox2b\ KO}$ mice and their littermate controls. As expected, all mice demonstrated increases in EE after exposure to HFD (Figures 3G–3L). However, the increases in oxygen consumption (Figure 3J) and heat production (Figure 3L) were markedly higher in PPARγ$^{Phox2b\ KO}$ mice compared to the controls.

BAT is the major thermogenic organ in rodents and humans (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Different from the white adipocytes, brown adipocytes contain many smaller lipid droplets and a much higher number of mitochondria that are needed for heat production (Harms and Seale, 2013). We examined the expression of mitochondrial genes that are important for BAT thermogenesis and found a mild but significant increase in uncoupling protein 1 (*Ucp1*) expression in HFD-fed PPARγ$^{Phox2b\ KO}$ mice (Figure 4A). Surprisingly, we found markedly increased expression of *Ucp1*, as well as other brown adipocyte markers, in the subcutaneous white fat depot of these mice (Figure 4B). Consistent with alterations in gene expression, we found that the subcutaneous white adipocytes of HFD-fed PPARγ$^{Phox2b\ KO}$ mice had adopted a new morphology (Figures 4C and 4D) and biomarker expression (Figures 4E and 4F) that were characteristic of brown adipocytes. Collectively, this suggests a mechanism by which the increased EE observed in PPARγ$^{Phox2b\ KO}$ mice involves the active reprograming of white adipocytes toward a brown adipocyte cell fate. Since sympathetic inputs play important roles in both the browning of white adipocytes (Schulz et al., 2013) and diet-induced thermogenesis (Bachman et al., 2002), we examined the expression of β3-adrenergic receptor in brown and white adipocytes and found that their levels were significantly elevated in PPARγ$^{Phox2b\ KO}$ mice (Figures 4A and 4B).

PPARγ Regulates Gene Expression in the NG

At least two alternatively spliced PPARγ isoforms have been identified in mice and humans. Unlike the widely expressed PPARγ1 isoform, PPARγ2 expression is largely restricted to adipose tissues (Tontonoz et al., 1994). Using isoform-specific probes, we found that NG neurons predominantly express the PPARγ2 isoform, and its levels were negatively regulated by HFD feeding (Figure 4G). PPARγ directs a transcriptional program that governs lipid synthesis and storage in adipocytes; however, its neuronal functions remain largely unexplored. To obtain molecular insights into how PPARγ modulates neurophysiology in vagal sensory neurons, we conducted genome-wide gene expression analysis and compared the transcriptomes of laser-captured NG neurons of control and PPARγ-deficient mice. Our analysis identified hundreds of differentially expressed genes (fold change ≥ 2; $p < 0.01$) in PPARγ-deficient NG neurons. We then verified the changes of selected genes by Taqman qPCR and found significant reduction of mRNAs of several PPARγ canonical transcriptional targets including *CD36*, *Lpl*, and *aP2* in PPARγ-deficient NG neurons (Figure 4H). Moreover, their expression in NG neurons was negatively regulated by HFD in wild-type mice, but not in the PPARγ$^{Phox2b\ KO}$ mice (Figure S1C). In addition to genes involved in lipid metabolism, we found many neuronal genes, including *Synt4, Gria2, Gria3,* and *Ntrk2*, that are important for transmitter release, synaptic transmission, and plasticity were also altered in PPARγ-deficient NG neurons (Figure 4I). While most of the differentially expressed genes were downregulated in PPARγ-deficient neurons, we found a small number of genes which were upregulated (Figure 4I). These include receptors for the anorexigenic peptide cholecystokinin (CCK) (Moran and McHugh, 1982). To test whether changes in gene expression have a physiological impact on vagal-mediated behavior, we measured the food intake in overnight-fasted control and PPARγ$^{Phox2b\ KO}$ mice following an acute intraperitoneal dose of saline or CCK. We found no differences in the amount of food intake when these mice were treated with saline (data not shown), whereas PPARγ$^{Phox2b\ KO}$ mice had an enhanced anorexic response to CCK and consumed significantly less food during the refeeding phase (Figure 4J).

DISCUSSION

The molecular mechanisms underlying nutrient sensing by the vagus nerve have been difficult to elucidate. This is due in large part to a relative lack of knowledge of the specific genes expressed in these neurons and the genetic tools that allow for their manipulation in vivo. Our findings that vagal sensory neurons express several NRs, including PPARγ, that can directly interact with dietary-derived lipids provide insights into how vagal afferents survey metabolic information from peripheral tissues and gate this information to the brain. It remains to be determined how PPARγ physically interact with fatty acids (FAs) within vagal

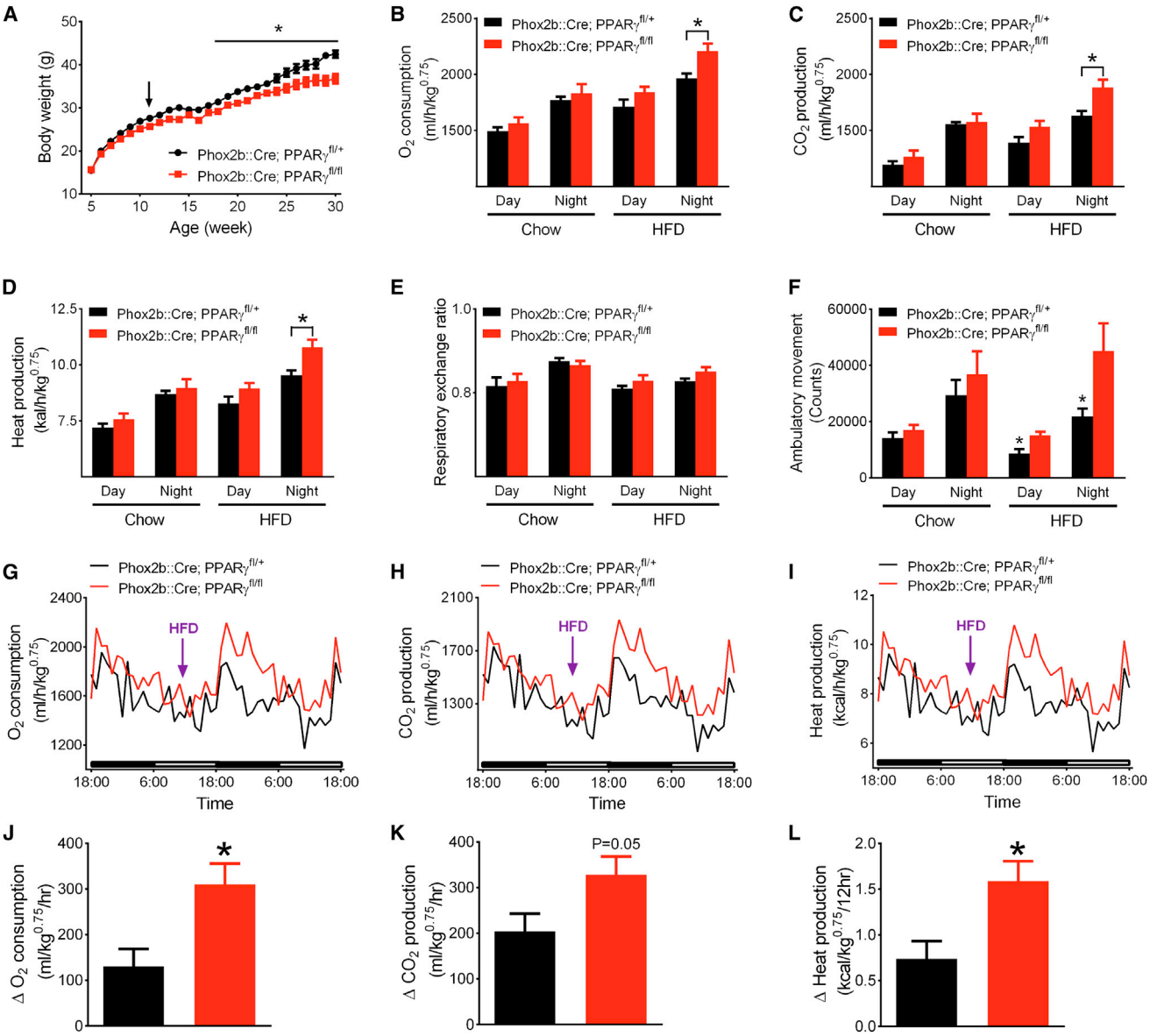

Figure 3. PPARγ in *Phox2b* Neurons Regulates Energy Expenditure

(A) Body weight of PPARγ$^{Phox2b\ KO}$ mice and littermate controls on chow diet were followed over time (n = 11 per genotype). Arrow indicates the age at which mice were tested in metabolic cages.

(B–E) Energy expenditure in weight-matched mice.

(B) O_2 consumption.

(C) CO_2 production.

(D) Heat production.

(E) Respiratory exchange ratio.

(F) Locomotor activity.

(G–L) Response to acute HFD challenge. Traces of O_2 consumption (G), CO_2 production (H), and heat generation (I) during 12 hr light and dark cycles.

(J–L) Changes in O_2 consumption (J), CO_2 production (K), and heat production (L) of the same mice before and after the switch to HFD. Error bars show SEM. The symbol * indicates $p < 0.05$.

sensory neurons. It will be intriguing to investigate whether PPARγ shuttles from the nucleus to vagal sensory terminals to directly capture circulating FAs. Alternatively, a second route may involve vagal sensory neurons' specialized ability to process FAs via the expression of several "adipocyte" genes. For example, the uptake of extracellular lipoproteins and long-chain FAs can be mediated by the scavenger receptor CD36 (Calvo et al., 1998). Moreover, different lipoproteins can be processed by lipoprotein lipases so that FAs are released and further targeted to the nucleus by adipocyte protein 2 (aP2) (Tan et al., 2002). We found that all these genes are expressed in vagal sensory neurons and that their levels were markedly reduced in

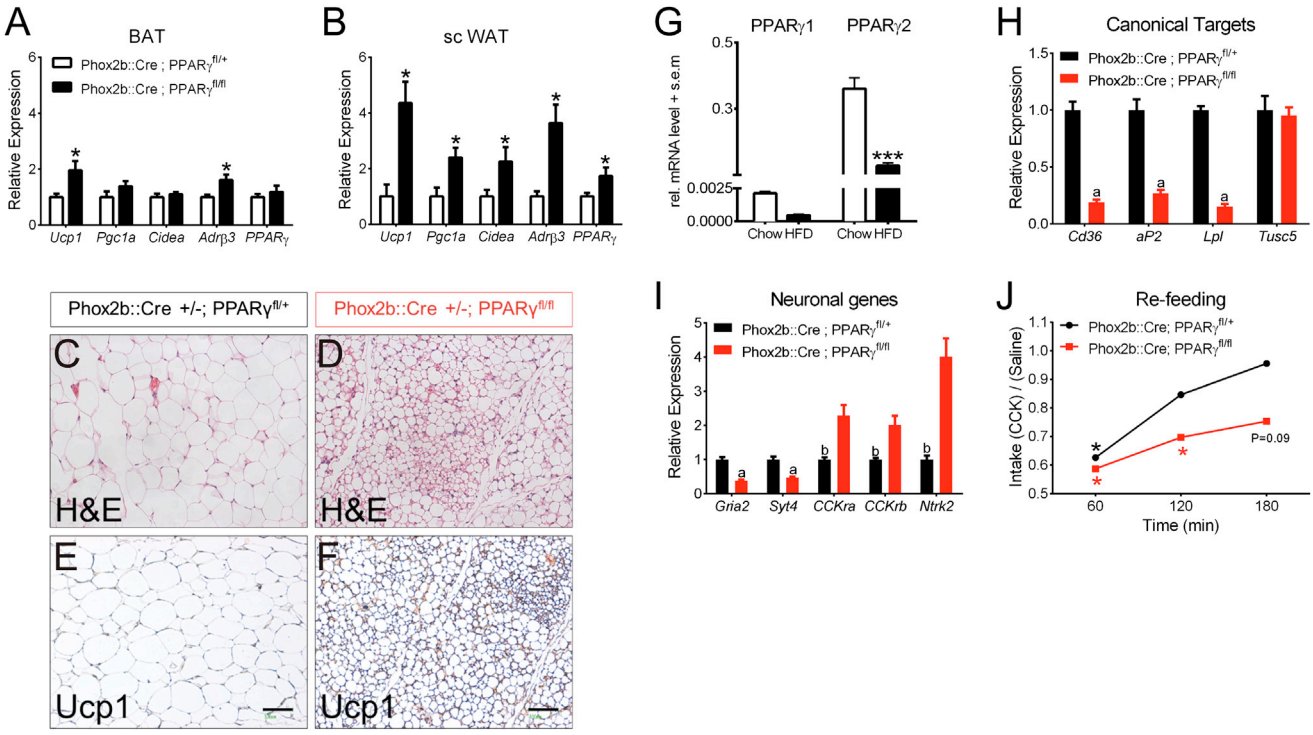

Figure 4. White Adipocyte Reprogramming and Altered NG Gene Expression in PPARγ$^{Phox2b\ KO}$ Mice

(A) Relative gene expression in BAT (n = 6 per genotype).

(B) Relative gene expression in subcutaneous. WAT (n = 6 per genotype). Error bars show SEM. The symbol * indicates $p < 0.05$.

(C and D) HE staining of subcutaneous WAT.

(E and F) Immunohistochemistry for UCP1 in subcutaneous WAT. Scale bar is 50 μm.

(G–J) PPARγ regulates gene expression in the NG.

(G) Expression of PPARγ1 and PPARγ2 mRNA in the NG of wild-type mice fed with either chow or HFD. The symbol *** indicates $p < 0.001$.

(H) Relative expression of several canonical PPARγ transcriptional targets expression in the NG.

(I) Relative expression of neuronal gene expression in the NG. *a* indicates $p < 0.01$; *b* indicates $p < 0.05$.

(J) Anorectic responses to CCK treatment in PPARγ$^{Phox2b\ KO}$ and control mice. Data are shown as percentage of food intake (CCK)/food intake (Saline). n = 11 to 13; * indicates $p < 0.05$ comparing food intake in the same animals treated with saline and CCK.

PPARγ-deficient NG neurons. Altogether, our data suggests that PPARγ directs a transcriptional program that governs the vagal sensing of FAs within NG neurons.

Similar to its function in adipocyte differentiation and lipid storage, we found that PPARγ plays an anabolic role in vagal neurons. Under normal dietary conditions, PPARγ acts to suppress EE, whereas upon acute exposure to HFD, its downregulation in vagal sensory neurons promotes diet-induced thermogenesis and may serve as an early protective mechanism against HFD-induced weight gain. These results are reminiscent of recent findings that mice lacking PPARγ in all neurons were also protected from HFD-induced obesity, but due to a combination of reduced feeding and increased EE (Lu et al., 2011). We hypothesize that the absence of a feeding phenotype in PPARγ$^{Phox2b\ KO}$ mice is likely due to the fact that our manipulations spare PPARγ function in other (e.g., hypothalamic) neurons where it regulates food intake (Diano et al., 2011; Ryan et al., 2011). On the other hand, our findings demonstrate that vagal neurons serve as a critical site where PPARγ regulates EE. Interestingly, our observation that the EE increase was further exacerbated in HFD-fed PPARγ$^{Phoxth2b\ KO}$ mice suggests the involvement of additional factors during diet-induced thermogenesis. Therefore, further studies are warranted to determine whether other NRs may also contribute to the same process and play a role in the vagal control of nutrient sensing and energy balance. Moreover, it would be intriguing to study whether the functional outputs of different NR programs in vagal afferents are segregated and responsible for different aspects of vagal-mediated behaviors (e.g., PPARα for food intake) (Tellez et al., 2013).

Many of the white adipocytes in PPARγ$^{Phox2b\ KO}$ mice adopted new morphologies and expressed biomarkers characteristic of thermogenic brown adipocytes. We suspect that increased sympathetic activity may underlie the browning of white adipocytes (Cousin et al., 1992), as evidenced by a significant increase in β3-adrenergic receptor expression in both BAT and subcutaneous WAT. Since both WAT and BAT receive innervation from sympathetic, but not parasympathetic afferents (Bowers et al., 2004), the increased EE in PPARγ$^{Phox2b\ KO}$ mice is likely the result of an increased sympathetic tone that is secondary to altered input from the parasympathetic vagal sensory neurons. The parasympathetic and sympathetic nervous systems interact at multiple levels along the neural axis. For example, NTS neurons not only receive and integrate metabolic signals from vagal afferents but also regulate sympathetic output through relay

circuits within the ventrolateral medulla (Grill and Hayes, 2012). Therefore, it is formally possible that PPARγ deletion in other *Phox2b* neurons including those in the NTS may contribute to the observed responses. However, recent studies suggest that EE can be directly affected by altered vagal sensory inputs from the periphery. For example, direct intraduodenal infusion of lipids increases EE in rats, a phenomenon that can be blunted by blocking neurotransmission of intestinal vagal afferents (Blouet and Schwartz, 2012). Similarly, Uno et al. (2006) reported that the increased EE by hepatic gene manipulation can be abolished by caspasin-mediated blockage of vagal afferents.

Despite its well-documented role in governing the transcription of genes important for lipid synthesis and storage, little is known about how PPARγ functions in neurons to regulate neurophysiology. Our analysis demonstrated that PPARγ regulates the expression of its canonical transcriptional targets, including *Lpl*, *CD36*, and *aP2*, in the NG neurons. Both Lpl and CD36 are present in discrete neuronal populations within the CNS (Glezer et al., 2009). Moreover, neuron-specific deletion of *Lpl* in mice causes profound defects in energy balance and cognitive behaviors (Wang et al., 2011). Importantly, presynaptic deficits were noted in *Lpl*-deficient hippocampal neurons that include the depletion of synaptic vesicles as well as a reduction in presynaptic gene expression (Xian et al., 2009). Our analysis also revealed a significant reduction in the expression of *Gria2*, a key component of AMPA receptors, which has been implicated in controlling glutamate release at presynaptic terminals of sensory afferents (Pinheiro and Mulle, 2008). Moreover, mRNAs of synaptotagmin-4, an important modulator for transmitter exocytosis (Zhang et al., 2011), was also reduced in PPARγ-deficient NG neurons. Our findings suggest the intriguing possibility that defective lipid sensing in vagal sensory neurons may lead to altered synaptic transmission. However, it remains to be determined whether the reduction of these presynaptic regulators is secondary to an *Lpl* deficiency or whether PPARγ directly regulates their transcription in NG neurons.

Finally, we found that PPARγ$^{Phox2b\ KO}$ mice have an enhanced anorectic response to CCK administration, suggesting that increased CCK receptor expression in PPARγ-deficient NG neurons affects vagal-mediated feeding behavior. In contrast, we found no overall changes in food intake or meal patterns in PPARγ$^{Phox2b\ KO}$ mice despite the upregulation of CCK receptors. This could be due to a compensatory mechanism triggered by the embryonic deletion of PPARγ in these mice. Similar observations were also made in *CCKra* null mice, which showed normal food intake despite a blunted anorexic response to acute CCK treatment (Kopin et al., 1999). In addition to its anorexigenic action, CCKra activation is required for the increase in EE seen after direct intraduodenal lipid infusion (Blouet and Schwartz, 2012). Interestingly, we found a conserved PPAR response element in the upstream regulatory sequences of *CCKra* gene (−396 bp upstream of exon 1), raising the possibility that PPARγ directly regulates its transcription in vagal sensory neurons.

Altogether, our studies uncover a role for PPARγ in vagal neurons in sensing and relaying information of peripheral lipid status toward the CNS. These findings further demonstrate that PPARγ-dependent lipid sensing by the vagus nerve is critical for the maintenance of long-term energy balance. The fact that vagal sensory neurons reside outside the blood-brain barrier makes them potential targets for a number of pharmacological agents. Moreover, there is emerging evidence that many antidiabetic and antiobesity agents directly act on vagal afferents, including agonists for the glucagon-like peptide 1 receptor and cannabinoid 1 receptor (Burdyga et al., 2004; Vahl et al., 2007). Therefore, studies on the roles of PPARγ as well as other NRs in vagal afferents will likely be a critical first step in developing novel vagal-based antiobesity and antidiabetic therapies.

EXPERIMENTAL PROCEDURES

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee at UT Southwestern Medical Center. Male mice heterozygous for the floxed PPARγ allele and hemizygous for the *Phox2b*::*Cre* transgene were crossed with female mice homozygous for the floxed PPARγ allele to generate Phox2b::Cre ± ; PPARγ$^{fl/fl}$ mice.

Laser Capture Microdissection

Nodose ganglia were cryosectioned (25 μm) using a cryostat and thaw-mounted onto saline-coated PEN membrane glass slides (Applied Biosystems, Foster City). Slides were lightly fixed in 75% ethanol immediately prior to thionin staining. Sections were then dehydrated in a series of graded ethanol baths followed by 5 min in xylenes. The Arcturus Veritas Microdissection System (Applied Biosystems) was used to isolate the neuron-enriched samples from the surrounding fibers and glial cells. RNA was extracted using a PicoPure RNA Isolation Kit (Applied Biosystems) with an on-column DNase I treatment to remove genomic contamination (QIAGEN, Valencia).

In Situ Hybridization

In situ hybridization was performed as described previously (Vianna et al., 2012). The following primers were used to generate S^{35}-UTP labeled riboprobes for LXRα (5′ GGATTCtgccatcagcatcttctctg 3′; 5′ ccgCTCCAGtctc ctggctagtttatttgg 3′), LXRβ (5′ GGATCCtgcagcagccatacgtgg 3′; 5′ ccgCTC GAGtaacctctctccactcaagg 3′), PPARα (5′ AATCAGAGATGCCACCTTGG 3′; 5′ GGGGTCAAAGGCTGGTTAAT 3′), and PPARγ (5′ CAGTGATATCGACCA GCTGAA 3′; 5′ CATGAATCCTTGGCCCTCT 3′).

Metabolic Cage Studies

Food intake, meal patterns, EE, and physical activity were continuously monitored using a combined indirect calorimetry system (TSE Systems GmbH, Bad Homburg, Germany). Experimental animals (11 weeks old) were acclimated in the metabolic chambers for 5 days before data collection. Mice were initially maintained on chow diet during the acclimation and in the first 2 days of data collection and then fed HFD for the next 3 days in the metabolic cages. O_2 consumption and CO_2 production were measured to determine the EE. In addition, physical activity was measured using a multidimensional infrared light bean system. Differences in EE before and after the switch to HFD were calculated by subtracting the total EE of 24 hr before the switch from that of 24 hr after the switch in each individual animal. All mice demonstrated increases in EE after exposure to HFD. After the metabolic cage analysis, mice were returned to their housing cages and fed again with chow diet.

Food Restriction

Weight-matched experimental animals (n = 11, per genotype; 15 weeks old) were singly housed for 7 days before the onset of food restriction, during which body weight and food intake were measured daily. The average daily intake during the 7 day period was then calculated for each individual mouse. Following an overnight fast, mice were refed with only 60% of the amount of their average daily consumption. Body weights and glucose levels were monitored every single day during the restriction. Food restriction was carried out for 7 days or terminated when individual mice lost more than 20% of their original body weight according to the institutional guidelines.

CCK Administration

Experiments were performed as previously described by Fan et al. (2004). Briefly, weight-matched PPARγ$^{Phox2b\ KO}$ mice (n = 11) and their littermates (n = 13) were used to test food intake in response to CCK-8 (Tocris, 10 μg/kg, intraperitoneally) or saline after fasting for 16 hr with water available. Thirty minutes after the injection, chow pellets are given to the singly housed mice, and food intake was measured hourly for the next 6 hr.

Data Analysis

The data were represented as mean ±SEM as indicated in each figure legend. Statistical analyses were performed using GraphPad PRISM version 6.0 (GraphPad, San Diego). After confirming normal distribution of data, comparisons between two genotypes were made by the unpaired Student's t test. Two-way ANOVA analyses were used to assess the interactions between genotypes and treatments with relevant post hoc tests. Statistical analysis in RT-qPCR experiment was carried out by using pairwise fixed reallocation randomization test. $p < 0.05$ was considered to be statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.01.021.

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